

## Ceftriaxone-Resistant *Neisseria gonorrhoeae*, Japan

**To the Editor:** Spread of multi-drug-resistant *Neisseria gonorrhoeae* is a major public health concern. Effective antimicrobial therapy is a key element in gonorrhea control. However, *N. gonorrhoeae* has developed resistance to multiple classes of antimicrobial drugs, including  $\beta$ -lactams, tetracyclines, and fluoroquinolones (1–3). Even an extended-spectrum oral cephalosporin-resistant, cefixime-resistant *N. gonorrhoeae* has emerged, and cefixime has now been withdrawn from use in Japan. Best practice treatment is limited to injectable extended-spectrum cephalosporins, such as ceftriaxone and spectinomycin. The emergence of ceftriaxone-resistant *N. gonorrhoeae* threatens effective disease control.

We identified a novel ceftriaxone-resistant *N. gonorrhoeae* isolated from a 31-year-old female commercial sex worker; MIC of ceftriaxone for this isolate was high (2  $\mu\text{g/mL}$ ). The woman visited a clinic in Kyoto for a routine examination for sexually transmitted infections in January 2009. Although she had no obvious symptoms or signs, a throat sample collected on her first visit yielded a positive result for *N. gonorrhoeae* by the strand displacement amplification test (ProbeTec ET, Becton Dickinson, Franklin Lakes, NJ, USA), but a vaginal sample taken at the same time was negative. After 2 weeks, another throat sample was positive for *N. gonorrhoeae* when cultured on Thayer-Martin medium, and the patient subsequently received 1 g ceftriaxone intravenously. Her pharyngeal sample was also *N. gonorrhoeae* positive by strand displacement amplification test on the third visit 2 weeks later, and further ceftriaxone treatment was prescribed. However, a culture for test of cure was not conducted because reinfection was

considered. A negative result was finally obtained in April 2009.

The culture showed positive reactions in oxidase and catalase tests. Gram staining showed gram-negative diplococci. The ID-test HN-20 Rapid system (Nissui, Tokyo, Japan) classified the bacterium as *N. gonorrhoeae*. Susceptibility was determined by the agar dilution method (4). For this strain, named H041, MIC of ceftriaxone was high (2  $\mu\text{g/mL}$ ), and the strain was highly resistant to penicillin G (4  $\mu\text{g/mL}$ ), cefixime (8  $\mu\text{g/mL}$ ), and levofloxacin (32  $\mu\text{g/mL}$ ). However, it demonstrated susceptibility to spectinomycin (16  $\mu\text{g/mL}$ ) and reduced susceptibility to azithromycin (0.5  $\mu\text{g/mL}$ ).

To characterize the ceftriaxone-resistant *N. gonorrhoeae* H041, multilocus sequence typing characterized the strain as ST7363 (5), which is the predominant sequence type (ST) among cefixime-resistant clones (6). *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) was also performed (7). The NG-MAST strategy uses 2 genes, *por* and *tbpB*, for porin and a transferrin-binding protein, respectively. NG-MAST indicated that the strain H041 was ST4220 and contained the *por*2594 allele and the *tbpB*10 allele. NG-MAST 4220 is a novel ST. However, the *tbpB*10 allele is the most frequently observed allele (76.5%) among multilocus sequence

typing-ST7363 *N. gonorrhoeae* strains ( $n = 81$ ) (M. Ohnishi, unpub. data).

Molecular typing suggested that the novel ceftriaxone-resistant *N. gonorrhoeae*, H041, is closely related to the ST7363 cefixime-resistant *N. gonorrhoeae*. Therefore, we compared *SpeI*-digested genomic DNA banding patterns of strain H041 with those of other *N. gonorrhoeae* strains by using pulsed-field gel electrophoresis as described (8). Four ST7363 strains, including *N. gonorrhoeae* H041, and 4 ST1901 strains (another major ST among cefixime-resistant *N. gonorrhoeae* strains) (6) were analyzed. The banding pattern of *SpeI* digested H041 genomic DNA was similar to that of other ST7363 strains and indistinguishable from that of cefixime-resistant but ceftriaxone-susceptible NG0207 (Figure).

We describe the emergence of ceftriaxone-resistant *N. gonorrhoeae*, isolated from a pharyngeal specimen from a female commercial sex worker. At 2  $\mu\text{g/mL}$ , the MIC was 4-fold higher than that of the previously reported ceftriaxone nonsusceptible strain (9). Our susceptibility testing suggests that only azithromycin and spectinomycin are effective drugs for treating this strain. In this case, eradication was successful, although *N. gonorrhoeae* colonization of the pharynx may just be temporary because

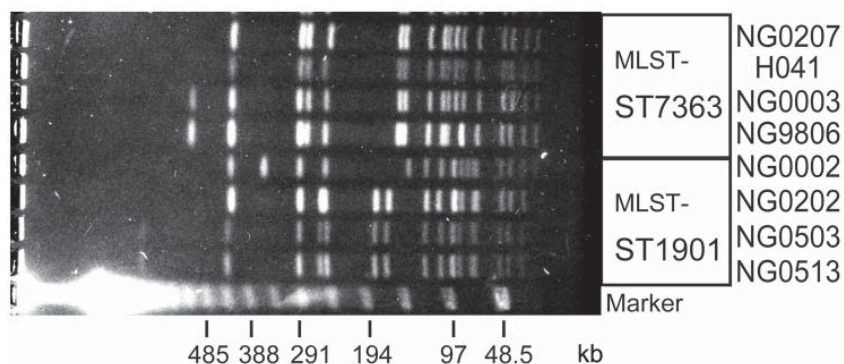


Figure. Pulsed-field gel electrophoresis patterns of ceftriaxone-resistant *Neisseria gonorrhoeae* strain H041 and other multilocus sequence typing (MLST) ST7363 and ST1901 strains. *SpeI*-digested genomic DNA from ceftriaxone-resistant *N. gonorrhoeae* H041, 3 of the MLST ST7363 strains and 4 of the MLST ST1901 strains were analyzed by pulsed-field gel electrophoresis. A lambda ladder standard (Bio-Rad, Hercules, CA, USA) was used as a molecular size marker.

the pharynx is not an ideal site for *N. gonorrhoeae* growth. From the routine examinations of commercial sex workers during January–March 2009, 40 *N. gonorrhoeae* were isolated in the clinic, but no other ceftriaxone-resistant strains were isolated. There is no evidence of dissemination of this strain in Kyoto.

Three independent molecular subtyping methods indicated that the ceftriaxone-resistant H041 strain was *N. gonorrhoeae*, and it might originate from an ST7363 cefixime-resistant *N. gonorrhoeae* clone. There are several possible mechanisms for the acquisition of resistance, including formation of a new mosaic type *penA* allele as *penA-X* cefixime resistance and acquisition of an extended-spectrum  $\beta$ -lactamase gene. The H041 strain did not produce  $\beta$ -lactamase in a nitrocephin test. Further molecular analysis is needed to elucidate the precise mechanism of the ceftriaxone resistance of the H041 strain.

The emergence of ceftriaxone-resistant *N. gonorrhoeae* raises concerns for controlling gonorrhea because ceftriaxone is widely recommended and the first-line treatment for gonorrhea around the world. *N. gonorrhoeae* has a potential to gain an extraordinarily high MIC to ceftriaxone. Surveillance for ceftriaxone-resistant *N. gonorrhoeae* should be strengthened.

#### Acknowledgment

We thank Hiroko Matsuoka for her technical assistance.

This study was supported by grants-in-aid from the Ministry of Health, Labour and Welfare of Japan (H21-Shinko-Ippan-001, and –012).

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DOI: 10.3201/eid1701.100397

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## Role of National Travel Health Network and Centre Website during Pandemic (H1N1) 2009

**To the Editor:** The National Travel Health Network and Centre (NaTHNaC) was created in 2002 by the Department of Health in England to provide authoritative guidance in travel medicine. The open-access NaTHNaC website ([www.nathnac.org](http://www.nathnac.org)) is a key mode of communication, with both health professionals' and travelers' areas. Website country information pages (CIP) provide specific guidance for travel to each country of the world, and an outbreak surveillance database (OSD) detailing global outbreaks of disease is updated daily.

In late April 2009, influenza A virus (H1N1) of swine origin was identified in 2 children from California, USA (1). These cases were traced to travel to Mexico, and a widespread outbreak of influenza A (H1N1) in Mexico subsequently was recognized. On June 11, 2009, the World Health